Low-dose Photon and Simulated Solar Particle Event Proton Effects on Foxp3$^+$ Treg Cells and Other Leukocytes

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Purpose: Radiation is a major factor in the spaceflight environment that can compromise immune defense mechanisms. Astronauts on missions are continuously exposed to low-dose/low-dose-rate (LDR) radiation and may receive relatively high doses during a solar particle event (SPE) that consists primarily of protons. However, there are very few reports in which LDR photons were combined with protons. The goal of this study was to determine whether exposure to LDR γ-rays would modulate the effect of proton radiation mimicking an SPE.

Materials and Methods: C57BL/6 mice were exposed to 1.7 Gy simulated SPE (sSPE) protons over 36 hours, both with and without pre-exposure to 0.01 Gy LDR γ-rays at 0.018 cGy/h. Apoptosis in skin samples was determined by immunohistochemistry immediately post-irradiation (day 0). Spleen mass relative to body mass, white blood cells (WBC), major leukocyte populations, lymphocyte subsets (T, Th, Tc, B, NK), and $CD4^+CD25^+Foxp3^+$ T regulatory (Treg) cells were analyzed on days 4 and 21.

Results: Apoptosis in skin samples was evident in all irradiated groups; the LDR+sSPE mice had the greatest expression of activated caspase-3. On day 4 post-irradiation, the sSPE and LDR+sSPE groups had significantly low WBC counts in blood and spleen; low lymphocyte levels were the most striking ($p < 0.05$ vs. 0 Gy). $CD4^+CD25^+Foxp3^+$ Treg cell numbers in spleen were decreased, but proportions were increased in the LDR and LDR+sSPE groups ($p < 0.05$ vs. 0 Gy). By day 21, lymphocyte counts were still low in spleens from the LDR+sSPE mice, especially $CD8^+$ T cytotoxic and natural killer (NK) cells, whereas other measurements were similar among groups.

Conclusions: The data demonstrate that pre-exposure to LDR photons did not protect against the adverse effects of radiation mimicking a large solar storm. The increased proportion of immunosuppressive $CD4^+CD25^+Foxp3^+$ Treg and persistent reduction in circulating lymphocytes may adversely impact immune defenses that include removal of sub-lethally damaged cells with carcinogenic potential, at least for a period of time post-irradiation.

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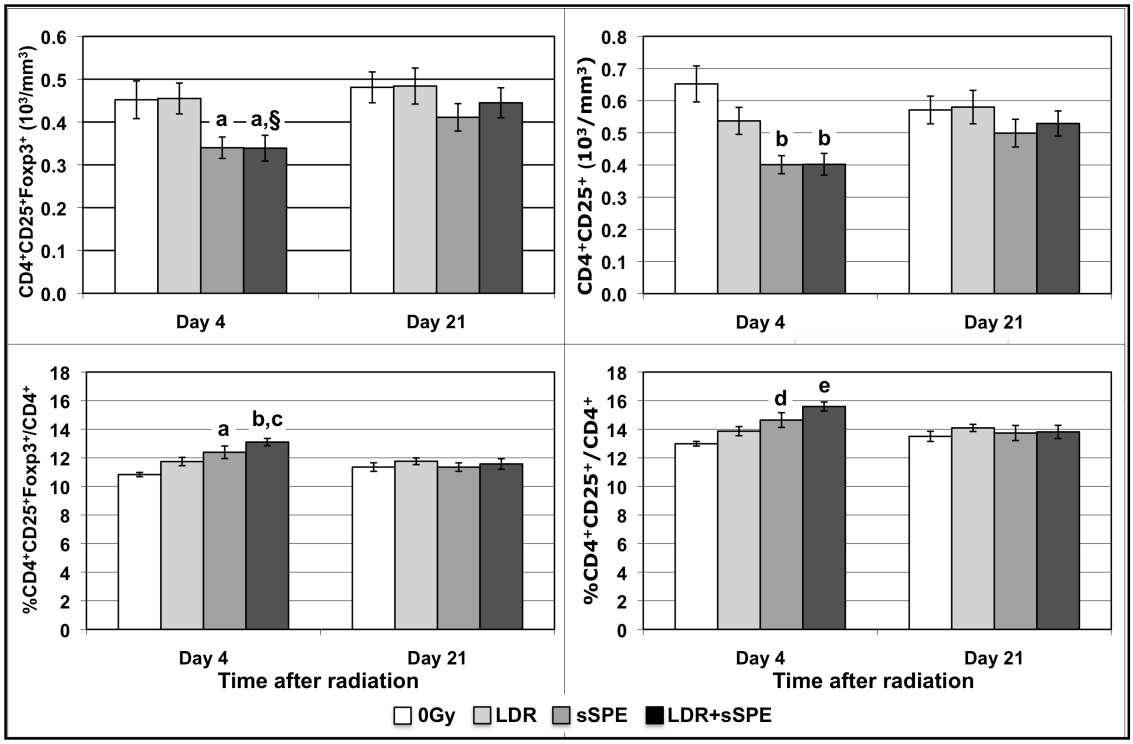
[Note: Selected data are presented on the next page.]

Percentages of Major Lymphocyte Populations in Blood and Spleen

Source & Group	Day 4*			Day 21**		
	T cells	B cells	NK cells	T cells	B cells	NK cells
Blood						
0 Gy	53.0±3.1	36.8±3.2	10.1±0.8	39.6±1.0	50.5±1.2	9.9±0.8
LDR	52.0±3.1	40.2±3.3	7.8±0.3	41.3±1.4	49.9±1.7	8.8±0.7
sSPE	62.5±2.8[#]	26.1±3.3[b,†]	11.4±1.2[b]	42.0±1.2	48.5±1.7	9.5±0.7
LDR+sSPE	61.1±2.0	24.5±2.2[a,c]	14.3±1.0[e,f]	44.1±1.3	45.1±2.0	10.8±0.9
Spleen						
0 Gy	37.1±0.9	58.7±0.8	4.2±0.2	37.5±0.7	58.4±0.8	4.1±0.3
LDR	36.9±0.5	58.7±0.7	4.4±0.2	36.2±0.9	59.4±1.0	4.3±0.5
sSPE	40.9±1.2[a]	52.7±1.3[d,e]	6.5±0.2[d,e]	37.5±1.1	57.9±1.3	4.6±0.5
LDR+sSPE	40.7±1.2[b,†]	52.5±1.0[d,e]	6.8±0.4[d,e]	37.6±0.9	58.4±1.1	4.0±0.3

Means ± SEM for n = 10-16 mice/group/time point. LDR: 0.01 Gy; sSPE, 1.7 Gy. *One-way ANOVA: $p < 0.05$ for %T, $p < 0.005$ for %B and $p < 0.001$ for %NK cells in blood; $p < 0.005$ for %T, $p < 0.001$ for %B and %NK cells in spleen. **One-way ANOVA: $p < 0.1$ for %T and %B cells in blood. a, $p < 0.0$ 5vs. 0 Gy; b, $p < 0.05$ vs. LDR; c, $p < 0.005$ vs. LDR; d, $p < 0.001$ vs. 0 Gy; e, $p < 0.001$ vs. LDR; f, $p < 0.01$ vs. 0 Gy; †, $p < 0.1$ vs 0 Gy; and #, $p < 0.1$ vs. LDR.

$CD4^+CD25^+$ T Regulatory (Treg) Cells and $CD4^+CD25^+$ T Cells in Spleen



Mean ± SEM for n = 10-16 mice/group/time point. Data were obtained using fluorescence-labeled monoclonal antibodies and flow cytometry. ANOVA (day 4), $p < 0.001$ for cells in all four panels. a, $p < 0.005$ vs. 0 Gy; b, $p < 0.001$ vs. 0 Gy; c, $p < 0.05$ vs. LDR; d, $p < 0.05$ vs. 0 Gy; e, $p < 0.01$ vs. LDR; §, $p < 0.1$ vs. LDR.